In the United States Patent and Trademark Office

Appn. Number:	10/687,272
Appn. Filed:	15 October 2003
Applicant(s):	Markus D. Herrema
Appn. Title:	Process for the Utilization of Ruminant Animal Methane Emissions
Examiner:	Rosanne Kosson
Art Unit:	1651

Mailed: 11 December 2004

At: Laguna Niguel, CA

Declaration Under Rule 132 Regarding Enablement

Commissioner for Patents
P.O. Box 1450
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Sir or Madam:

Markus D. Herrema declares as follows:

1. I am the inventor in the above patent application.

2. In the Office Action mailed 15 September 2004 regarding the above patent application, claims 1-17 were rejected under 35 U.S.C. § 112 since they were said to contain "subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most closely connected, to make and/or use the invention."

3. The Office Action noted in particular that "because the specification does not demonstrate that the claimed invention works," the skilled artisan "would not have been able to determine whether or not the claimed method is operational as described." In the absence of experimental data, it was said, the operability of the claimed invention as described came into question on four specific points.

4. **Summary of the Four Operability Questions Posed in the Office Action.** First, the Office Action asked, what is the concentration of methane exhaled by a ruminant animal needed to produce a grown culture of methane-utilizing microorganisms in the above-mentioned growth apparatus? Second, will methane-utilizing microorganisms existing in such a growth apparatus be able to grow using the methane exhaled by a ruminant animal as a source of carbon and/or energy? Third, would methane-utilizing microorganisms existing in the growth apparatus grow better if the breath of a ruminant animal were pumped into the apparatus than if methane-utilizing microorganisms were merely exposed to growth-culture medium and unadulterated (non-pumped) air? Fourth, would methane-utilizing microorganisms existing in the above-mentioned growth apparatus grow if the apparatus was mounted on a ruminant animal but the breath of the ruminant animal was not pumped into the apparatus?

5. **Purpose of the Declaration.** The purpose of this Declaration Under 37 C.F.R. § 1.132 is to present experimental data obtained through experimentation conducted by the applicant or under the personal supervision of the applicant, alongside knowledge pertaining to the state of the art of methane-utilizing microorganisms at the time the application was filed, to address each of these four operational points and thereby demonstrate and confirm that the claimed invention is operational as described.

6. **Outline of the Claimed Invention.** The claimed invention involves the utilization of the methane exhaled through ruminant animal exhalation as a source of energy,

whereby methane exhaled by a ruminant animal is captured, collected, and conveyed to a growth apparatus containing methane-utilizing microorganisms and a microorganism growth medium, thereby causing methane-utilizing microorganisms to grow in a growth-and-harvest apparatus using ruminant animal exhalation methane as a source of carbon and/or energy.

7. **I: The Concentration of Methane Needed to Practice The Claimed Invention.** To begin with the first question of operability posed in the Office Action, 'the concentration of methane needed to practice the claimed invention' is the same as 'the in-air concentration of methane exhaled through ruminant animal exhalation conveyed to a growth apparatus containing a growth-culture medium needed to grow methane-utilizing microorganisms.' It is well known in the art of methane-utilizing microorganisms that the in-air concentration of methane at which methane-utilizing microorganisms grow in nature ranges from 1.7 parts per million (the concentration of methane in the atmosphere) to approximately 500,000 parts per million (see Specification, "Background of the Invention—Prior Art," as well as Patel, et al. and Apel, et al. in Information Disclosure Statement for reference to the well-known art of methane-utilizing microorganisms). In isolated growth chambers containing only methane and a suitable nutrient substrate, methane-utilizing microorganisms can grow and reproduce using methane as a source of carbon and/or energy within the same methane concentration range: from 1.7 to 500,000 parts per million by volume (ppmv). While it was traditionally difficult to grow methane-utilizing microorganisms in isolation at atmospheric methane concentration levels (1.7 ppmv), the capacity to consistently grow methane-utilizing microorganisms at this concentration level has been known in the art since a study published in 1998 detailed how the use of small amounts of methanol as part of the growth-culture medium enabled methane-utilizing microorganisms to oxidize methane at atmospheric

concentration levels. In addition, "high affinity" methane-utilizing microorganisms have also been found to have the capacity to maintain growth at atmospheric methane concentration levels. As to recognized upper concentration limits, while methane-utilizing microorganisms are commonly incubated in culture conditions containing in-air methane concentrations of 500,000 ppmv, optimal conditions for methane-utilizing microorganism bioreactor growth have been shown to fall between 40,000 and 200,000 ppmv. Thus, to address the first operability question related to enablement listed in the Office Action, the concentration of methane exhaled through ruminant animal exhalation in air conveyed to a growth apparatus needed to grow methane-utilizing microorganisms, as would be understood by one skilled in the art of methane-utilizing microorganisms at the time that the application was filed, lies in the range between 1.7 and 500,000 ppmv.

8. **II: Methane-Utilizing Microorganism Growth From Methane Exhaled Through Ruminant Animal Exhalation.** The second question of operability related to enablement raised in the Office Action pertains to the capacity of methane-utilizing microorganisms to use, as a source of carbon and/or energy for growth, methane that has been exhaled by a ruminant animal and conveyed to a growth-and-harvest apparatus. Specifically, the Office Action stated, "[t]here is no evidence on the record, however, for example experimental data, that methane taken from a cow's breath produced a grown culture of a methylotrophic microorganism." In order to confirm that methane that has been exhaled by a ruminant animal and conducted to a growth-and-harvest chamber containing a growth-culture medium and methane-utilizing microorganisms will produce a grown culture of a methane-utilizing microorganism, three experiments consisting of eight culture tests were carried out, as detailed below.

9. Experiment I: Producing a Grown Culture of Methane-Utilizing Microorganisms Using Methane That Had Been Exhaled by a Ruminant Animal, Collected, and Conducted to a Growth Chamber Containing Methane-Utilizing Microorganisms and a Growth-Culture Medium. In Experiment I, methane-utilizing microorganisms were first isolated by taking four various soil samples, each approximately 5.0-20 grams (g) by weight, diluting these samples with distilled water, and adding approximately 50 milli-liters (ml) of each diluted sample into four separate 250 ml serum bottles. After approximately 50 ml of mineral salts medium were added to each bottle, each bottle was then sealed with a rubber septum. Each liter of mineral salts medium contained 1 g KH_2PO_4, 1 g K_2HPO_4, 1 g KNO_3, 1 g NaCl, 0.2 g $MgSO_4$, 26 mg $CaCl_2*2H_2O$, 5.2 mg EDTA $Na_4(H_2O)_2$, 1.5 mg $FeCl_2*4H_2O$, 0.12 mg $CoCl_2*6H_2O$, 0.1 mg $MnCl_2*2H_2O$, 0.07 mg $ZnCl_2$, 0.06 mg H_3BO_3, 0.025 mg $NiCl_2*6H_2O$, 0.025 mg $NaMoO_4*2H_2O$, 0.015 mg $CuCl_2*2H_2O$. Next, after natural gas was injected through the rubber septum of each airtight bottle via needled-syringe until the total concentration of methane in the headspace of each bottle was approximately 40,000 ppmv, the five airtight bottles were incubated at room temperature (22 degrees Celsius) in an inverted position. After seven days, the liquid and suspended liquid contents of each bottle were transferred in equal proportions into five new 250 ml serum bottles, and approximately 50 ml of fresh mineral salts medium, as described above, were then added to each of the five new "non-gassed" bottles. With only atmospheric air inside each of the five new bottles, each bottle was then sealed with a rubber septum as above, and a needled-syringe was used to draw all or most of the air out of each bottle. Next, atmospheric air and air that had been exhaled by a ruminant animal (including the methane contained therein) were simultaneously collected by drawing air surrounding the nose and mouth of a dairy cow into a plastic syringe (where the air-in tip of the syringe was placed approximately 1 inch from the nose and mouth of a standing dairy cow), and this exhaled/atmospheric air mixture was subsequently injected via needled-syringe in equal volumetric amounts into each of

the five septum-sealed bottles. Methane-utilizing microorganism growth was assessed in each of the five bottles containing methane exhaled by a dairy cow by monitoring methane concentration levels in the headspace of the five bottles 24 hours (1 day) and 288 hours (12 days) after the introduction of dairy cow exhalation gas to the bottles. Methane levels were measured via gas chromatography using a Hewlett-Packard Series II 5890-A Gas Chromatograph (GC), where helium was the carrier gas in a GC oven temperature of 200 degrees Celsius.

10. Experiment I: Results. Methane that had been exhaled by a dairy cow and conducted to a growth chamber containing methane-utilizing microorganisms and a growth-culture medium enabled the growth of methane-utilizing microorganisms in five separate serum bottles at methane degradation rates of 12, 10, 14, 14, and 16 percent (% decrease in headspace methane concentration), respectively, over a period of 12 days. In bottles 1 and 2, headspace methane concentration levels were degraded by 12% (from 602 ppmv to 532 ppmv) and 10% (from 546 ppmv to 490 ppmv), respectively, from initial concentration levels over a period of 12 days. In bottles 3, 4, and 5, headspace methane levels dropped by 14% (from 518 ppmv to 448 ppmv), 14% (from 504 ppmv to 434 ppmv), and 16%, (630 ppmv to 532 ppmv) respectively, from initial concentration levels over a period of 12 days. Thus, methane that was exhaled by a dairy cow, collected, and conveyed to a growth apparatus containing methane-utilizing microorganisms and a growth-culture medium was shown to effectively enable methane-utilizing microorganism growth.

11. Additional Experimentation/Experiment II: Simulating Potential Growth Conditions. While the experiment described above demonstrated the capacity of methane-utilizing microorganisms to grow in culture conditions where methane exhaled through ruminant animal exhalation was present in sufficient growth-enabling concentrations, additional experimentation was carried out to confirm the sustained growth capacity

of methane-utilizing microorganisms at methane concentration levels similar to those present in Experiment I as well as those to which methane-utilizing microorganisms might be exposed in the claimed invention.

12. As disclosed in the application, dairy cows emit up to 634 quarts of methane per cow per day through processes of exhalation, although the hourly volume of methane emissions vary, as disclosed in the specification, according to digestive activities (see Polakovic, "Getting the Cows to Cool It," *Los Angeles Times*, 2003 June 7, pp. A1 and A7, Los Angeles, CA U.S.A. in the Information Disclosure Statement for reference to dairy cow methane emissions rates; see "Background of the Invention--Prior Art" in the specification for reference to the known relationship between ruminant animal methane production and associated digestive activity). Although it is simple to calculate the average concentration of methane in dairy cow exhalation using the well-known knowledge of average dairy cow tidal volume, respiration rates, and methane exhalation emissions (the average methane concentration level per breath comes to approximately 4,000 parts per million), Experiment II simulated methane concentration levels according to the concentration levels at which methane-utilizing microorganisms grew in Experiment I—around 600 ppmv—where methane-utilizing microorganisms grew directly from methane that had been exhaled through dairy cow exhalation in a confined growth apparatus.

13. Upon completion of the twelve-day course of Experiment I, detailed above, approximately 20 ml of the liquid and suspended liquid contents of each of the five trial serum bottles, including the grown cultures of methane-utilizing microorganisms contained in the bottles, were transferred into two empty and unsealed 250 ml serum bottles. After 50 ml of fresh mineral salts medium, as described in Experiment I, and approximately 100 ml of transferred liquid contents had been added to each of the

two new bottles, each bottle was then sealed with a rubber septum. Next, natural gas was added via needled-syringe into each bottle until the headspace methane concentrations of bottles 6 and 7 were 1022 and 795 ppmv, respectively. After 17 days of inverted-position incubation at room temperature, approximately 50 percent of the liquid and suspended liquid contents of bottle 7 (including the grown culture of methane-utilizing microorganisms contained therein) were transferred to an empty 250 ml serum bottle. After approximately 75 ml of transferred solution and 50 ml of fresh mineral salts medium had been added, bottle 8 was sealed with a rubber septum, as above, and natural gas was added until the headspace methane concentration was 323 ppmv. Bottle 8 was incubated for 12 days in an inverted position at room temperature. As above, headspace methane concentration level degradation was measured via gas chromatography using a Hewlett-Packard Series II 5890-A Gas Chromatograph to assess methane-utilizing microorganism growth.

14. Experiment II: Results. Methane concentration levels similar to those found in Experiment I enabled the growth of methane-utilizing microorganisms in three serum bottles (containing methane-utilizing microorganisms and growth-culture media) at headspace methane concentration degradation rates of 94, 95, and 75 percent over a period of 17, 17, and 12 days, respectively. In bottles 6 and 7, headspace methane concentration levels were degraded by 94% (from 1022 to 70 ppmv) and 95% (from 795 to 39 ppmv), respectively, from initial concentration levels over a period of 17 days. In bottle 8, headspace methane concentration levels dropped by 75% (from 322 to 74 ppmv) from initial concentration levels over a period of 12 days. Thus, culture conditions similar to those carried out in Experiment I were also found to effectively enable methane-utilizing microorganism growth.

15. In total, between Experiment I and Experiment II, methane that had been exhaled by a ruminant animal, collected, and conveyed for use in a growth apparatus, or methane

that was available in concentrations similar to those found in Experiment I, enabled methane-utilizing microorganism growth over a course of 41 days.

16. **III: The Growth Advantage to Methane-Utilizing Microorganisms of Pumping Methane Exhaled by a Ruminant Animal Into a Growth Chamber (Versus Only Exposing Them to Unadulterated Air).** The third operability question posed in the Office Action pertained to the efficacy of pumping methane that had been exhaled through ruminant animal exhalation into the growth apparatus described. As the Office Action stated: "There is no evidence that if the apparatus described in the application, including one or more methylotrophic microorganisms and culture medium, were placed on a cow, that the methylotrophic microorganisms would grow better if a cow's breath were pumped into the chamber containing the microorganisms than if the chamber were exposed to unadulterated air."

17. There are two fundamental reasons why methane-utilizing microorganisms would grow better in a growth apparatus, as described above, where 'exhaled breath' was pumped into the apparatus than in the same apparatus exposed only to 'unadulterated air'. First, since methane exhaled through ruminant animal exhalation becomes more diffuse according to, among other factors, the amount of time that has passed following exhalation, while the average methane concentration of dairy cow breath may be 4,000 ppmv, this concentration will decrease as exhaled methane disperses in air. Since it is well known that methane-utilizing microorganisms grow better in higher methane concentration culture conditions than lower methane concentration culture conditions due to advantages of methane (and, thus, carbon) accessibility, air in which exhaled methane is less dispersed will enable higher methane-utilizing microorganism growth rates than air in which exhaled methane is more dispersed. Thus, since the concentration of methane exhaled by a ruminant animal will be highest in air near the nose and mouth of a ruminant animal (or in areas where

ruminant animal exhalation is either confined or directed) and less concentrated, comparatively, in air on the back of a ruminant animal, if the concentrated breath of a ruminant animal is pumped into a growth chamber containing methane-utilizing microorganisms and a growth-culture medium, it will enable higher microorganism growth rates than if the chamber is merely exposed to lower-concentration air.

18. However, secondly, even if the concentrations of methane in "exhaled breath" and "unadulterated air" were identical, methane-utilizing microorganisms would still grow better "if a cow's breath were pumped into the chamber" than if the chamber were merely exposed to "unadulterated air." To illustrate, if 1000 liters of "exhaled breath" with a methane concentration of 4000 ppmv were pumped into the growth chamber every hour, the total amount of methane pumped into the growth chamber would be 4 liters per hour, or 96 liters per day. If 1 liter of "unadulterated air" with the same methane concentration (4000 ppmv) passed through the same growth chamber every hour, the *total amount of methane* passing through the growth chamber, and, thus, the amount of methane available for methane-utilizing microorganism growth, would 1000 times less, at 0.096 liters per day, than the amount of methane passing through the 'pumped breath' chamber. Thus, even if the concentrations of exhaled methane in 'exhaled breath' and 'unadulterated air' are the same, directing or pumping air into the growth chamber described increases the total amount of methane passing through the growth chamber, thereby increasing the amount of carbon available for growth and enabling more or "better" methane-utilizing microorganism growth.

19. **IV: Growing Methane-Utilizing Microorganisms From Methane Exhaled Through Ruminant Animal Exhalation in Unadulterated Air.** The final question pertaining to the operability of the claimed invention relates to the capacity of methane-microorganisms to grow using methane exhaled through ruminant animal

exhalation in a growth chamber containing growth-culture medium when the growth chamber is situated on the back of a ruminant animal and exposed only to unadulterated or "non-pumped" air. As stated in the Office Action: "as no indication of the concentration of methane needed to practice the claimed invention is provided, there is no indication as to whether or not the microorganisms would grow if the chamber were simply mounted on the cow without the cow's breath pumped in."

20. If a growth chamber were mounted on the back of a ruminant animal for the purpose of using methane exhaled through ruminant animal exhalation as a source of carbon and/or energy to produce methane-utilizing microorganisms, this purposefully situated growth chamber would only be functional as described, though perhaps not highly efficient, if the elevated concentration of methane exhaled through ruminant animal exhalation in the air entering the growth chamber was sufficient to produce methane-utilizing microorganism growth. As detailed above, "the concentration of methane exhaled through ruminant animal exhalation in air conveyed to a growth apparatus needed to practice the claimed invention, as would be understood by one skilled in the art of methane-utilizing microorganisms at the time that the application was filed, lies in the range between 1.7 and 500,000 ppmv." Therefore, if 1) an apparatus as described in this paragraph and paragraph 19 were situated on the back of a cow for the purpose of causing methane-utilizing microorganisms to grow in a confined growth-and-harvest chamber by mutually-exposing methane-utilizing microorganisms, growth-culture media, and the methane exhaled by a ruminant animal and 2) the concentration of ruminant animal-exhaled methane in air entering the apparatus was consistently elevated to levels between 1.7 and 500,000 ppmv, this directed mutual-exposure would enable the growth of methane-utilizing microorganisms as described.

21. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. § 1001 and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Very respectfully,

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